

The University of Chicago

SERUM CALCIUM
AND HYPERPARATHYROIDISM
IN RICKETS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE PHYSICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

1933

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CHARLES SCHWARTZ

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The work herein reported was done under the direction of Professor Bengt Hamilton, for whose unfailing interest and constant advice and assistance the writer wishes to express his sincerest appreciation.



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INTRODUCTION¹

Rickets, as is well known, is a condition which occurs in the young animal organism, when it is subjected to a dietary lacking in vitamin D, and, in the case of the small laboratory animal, unbalanced in mineral content--especially with reference to calcium and phosphorus. This particular manifestation of a dietary deficiency is characterized by certain well defined lesions of the bones, and by abnormal values for certain constituents of the blood.

In 1920, Iversen and Lenstrup² pointed out that the acid soluble phosphorus in the serum of rachitic infants was below normal; and in 1921, Howland and Kramer,³ in a report covering a large number of cases showed that in active rickets the inorganic phosphorus of the serum is reduced below normal while the serum calcium is generally normal, although in some cases it is slightly lowered. Their observations have been substantiated by almost every investigator who followed them.

A few years later, the observation was made that if phosphates were given to rachitic infants, the serum phosphorus rose and the calcium correspondingly dropped.⁴ Hamilton and his

¹Some of the material reported in this paper has already been published: Hamilton and Schwartz, J. Pharm. and Exp. Ther. 46, 285 (1932); Am. J. Dis. of Child. (in press).

²Forhandlingerne ved Forste Nordiske Kongres for Paediatrici (1920) (Quoted from ³).

³Am. J. Dis. of Child. 22, 105 (1921).

⁴Orr, Holt, Wilkins, and Boone, Am. J. Dis. of Child. 28, 574 (1924).

coworkers¹ studied the effect of increasing the serum phosphorus of rachitic animals by various means and found that, in general, a rise in the concentration of the inorganic phosphorus of the serum, occasioned by "phosphorus administration, therapeutic agencies, or spontaneous healing," was accompanied by a drop in serum calcium. No one, however, except for a few experiments of Hamilton,² had, up to the time the present work was begun, studied the effect of the administration of soluble calcium salts to rachitic animals. Faber³ studied the effect of calcium chloride on rachitic infants, but inasmuch as the dosage he used was very small, about 0.20 gram per kilogram of body weight, and inasmuch as all of his cases had rickets complicated by tetany, the results which he observed are not a true representation of the reaction of animals in a pure rachitic condition to large doses of soluble calcium salts. It was with the view in mind of determining what the effect of such administration would be, that the present work was undertaken.

¹Hamilton, Kajdi, and Meeker, J. Biol. Chem. 88, 331 (1930).

²Unpublished data.

³Undersøgelser over Serumkalken og Serumfosforet Børnets forste livear, Nielson and Lydiche, Copenhagen, 1928.

PROCEDURE

Rabbits, about twenty-one days old, were placed on McCollum's rachitic diet 3143.¹ Control rabbits were put on the same diet to which had been added for every 100 grams of diet, one cubic centimeter of cod liver oil. After a period of three to four weeks, the animals were X-rayed. At that time those animals which were on the diet without cod liver oil, had developed typical rachitic symptoms as evidenced by X-ray photographs of the bones and by the inorganic phosphorus content of the serum. The controls, of course, were normal.

The animals were bled by heart puncture into a syringe containing a little mineral oil to prevent as much as possible the loss of carbon dioxide from the blood. About ten cubic centimeters were drawn. The blood was placed in an ice box for about one half hour to allow it to clot. The serum was separated by centrifugalization and analyses were made for serum inorganic phosphorus, serum calcium, and in some cases, pH. Within a period of from one hour to twenty-four hours after an animal had been bled for the first time, it was given by stomach tube ten cubic centimeters of a solution containing in most cases 100 milligrams of calcium in the form of calcium chloride or calcium gluconate. This salt solution was washed down with an additional five cubic centimeters of water. In five to fifteen minutes after this administration of calcium, the rabbit was bled as before, and similar analyses were made.

¹McCollum, Simmonds, Shipley, and Park, Proc. Soc. Exp. Biol. and Med. 19, 123 (1921).

No gross effects of the administration of calcium in this manner to normal rabbits could be observed. The effects on the rachitic animals were, however, very marked. Shortly after the calcium was given, the animals exhibited signs of extreme discomfort, and in many cases died within several minutes. The blood when drawn was very viscous and coagulated rapidly. The effects upon the serum constituents which were determined by analysis are shown in Tables I, II, III, and in Figure 1.

Table I shows the reactions of the normal rabbits to the doses of calcium chloride or calcium gluconate administered. The average value for the serum calcium of the normal rabbits is 3.09 millimols per liter of serum. The lowest value is 2.88 millimols per liter, and the highest is 3.30. The values after the administration of calcium are, as may be expected, slightly higher; the average being 3.94 millimols per liter, and the range being in all cases but one 3.20 millimols per liter to 4.23 millimols per liter. In one case, #16', the second calcium value was 4.70. Thus it may be seen (Table III) that in all of the twenty-three normal cases the serum calcium before the administration of calcium was between 2.69 and 3.39 millimols per liter. After the administration of calcium, the serum calcium rose in all cases to values between 2.69 millimols per liter and 4.70 millimols per liter, with only one above 4.49, and most of them in the range 3.40 to 4.49.

In Table II may be seen the results of the administration of calcium to rachitic rabbits. The average value of the serum calcium before the administration of calcium was 2.97 millimols per liter, which is very close to the average value for the controls. The range is 2.70 to 3.15 millimols per liter. The serum calcium values following the administration of calcium to rachitic

rabbits, however, are markedly different in that they rise to values much higher than those reached by the normal animals.

Table III which summarizes these results shows that of the thirty-three rachitic rabbits given calcium by mouth, all but seven have reached values for serum calcium concentration which exceed the highest value reached by the controls. In some cases, as may be seen, (Table II), the rachitic values were excessively high.

It is interesting to note that, although the increase of the inorganic phosphorus in rachitic rabbits' blood may cause a rather serious drop in the serum calcium,¹ the administration of calcium on the other hand, as may be seen from the data presented, causes little or no change in the inorganic phosphorus of the serum. In three cases, #93B, #76, and #6, there was a marked rise in the inorganic phosphorus of the serum, and a considerable drop in the pH. Inasmuch as this picture is frequently seen in moribund animals, these changes should be attributed to the poor condition of the animals (they died shortly after the blood was drawn), rather than to the hypercalcemia; hence these values were omitted in calculating the averages for the group.

In both the normal and rachitic animals there was a moderate drop in the pH of the blood after the administration of calcium. This decrease in pH was no more marked in one group than in the other, and was probably due to a rapid and rather complete absorption of the acid radical of the salt administered. It is well known that the administration of calcium chloride may result in an increased acidity of the blood.²

¹Hamilton, Kajdi, and Meeker, loc. cit.

²Gamble, Blackfan, and Hamilton, J. Clin. Invest. 1, 359 (1925).

Figure 1 summarizes in graphical form the changes in the composition of the blood serum resulting from the oral administration of calcium salts. It shows conclusively, together with the data presented in the tables, that rachitic serum, under the conditions of the described experiment, is able to take up more calcium than normal serum.

The reason for the tremendous avidity of the blood of rachitic animals for calcium is not easy to see from the data presented thus far. One of the things which might account for it is an increase in the acidity of the blood. However, as was shown above, the difference in the pH of normal and rachitic blood after the administration of calcium is not great enough to account for the marked difference in the calcium-holding capacities of the sera.

Another alternative is that the serum of rachitic animals may contain some substance which permits the solution of excessive amounts of calcium. That such a substance exists was shown by Collip.¹ He succeeded in preparing from the parathyroid glands of animals an extract which had the property of increasing the amount of calcium which the blood can hold. The possibility that this or a similar substance in the blood of rachitic animals was responsible for the results obtained, was augmented by a review of the literature dealing with the state of the parathyroid glands as associated in the organism with bone diseases,--especially rickets.

The first report concerning the state of the parathyroid glands in rickets was made by Erdheim.² He found that the size of the glands in rachitic rats was markedly increased, and stated

¹J. Biol. Chem. 63, 395 (1925).

²Dankschift d. Kaiserl. Akad. d. Wiss. 90, Vienna (1914)
(Quoted from ¹²).

that this increase in size was due to general hyperplasia of the glands and to individual hypertrophy of the cells. Ritter¹ examined the parathyroid glands of several rachitic children and found definite enlargement. Pappenheimer and Minor² in a similar study corroborated the findings of Ritter and stated, "that this enlargement is due to multiplication of cells, and not to an increase in the size of the cells." Nonidez and Goodale³ reported too, that the lack of anti-rachitic vitamin and the lack of direct sunshine on growing chicks manifested themselves, among other things, in an enlargement of the parathyroid glands.

Thus the work reported in the literature, together with the fact that the rachitic rabbits to which calcium had been given reacted very similarly to animals under the influence of parathyroid extract, pointed toward the plausibility of the hypothesis that in rickets there is a hyperactivity of the parathyroid glands.

In order to test this conjecture, it was necessary to develop a means of detecting small amounts of parathyroid hormone. The ordinary method of testing for the active principle of the parathyroid glands, which consists of determining its effect upon the serum calcium of a dog,⁴ could hardly be expected to be of use in this connection, due to the fact that comparatively large amounts are necessary in order to produce a demonstrable effect. However, in order to make this certain, a few experiments were performed. Blood from rachitic rabbits was injected into dogs and the serum calcium values were determined at intervals during a period of ten to twenty-four hours. As can be seen from the

¹Frankfurt Zeitschr. f. Path. 24, 137 (1920).

²J. Med. Res. 42, 391 (1920-21).

³Am. J. Anat. 38, 319 (1926).

⁴Collip and Clark, J. Biol. Chem. 64, 485 (1925)

data in Tables IV and V and in Figure 2, the results, although not negative, were not marked enough to be conclusive. The average rise in the serum calcium of the dogs resulting from the injection of normal rabbit blood was 0.07 millimols per liter, that caused by the injection of rachitic rabbit blood was 0.43 millimols per liter, and that occasioned by the injection of fifty units of parathyroid extract was 1.30 millimols per liter. Although the difference in the results of the injection of normal and rachitic blood into dogs is moderately large, the experiments presented are very few in number, and cannot be interpreted to show anything but promise of the results expected.

Inasmuch as continued experiments on dogs would require tremendous amounts of blood, it was thought that it might be possible to devise a suitable test for small amounts of parathyroid hormone in which rabbits could be used as the test animals. According to Bodansky, Blair, and Jaffe,¹ Collip,² and Greenwald and Gross,³ the injection of Parathormone⁴ into rabbits produces no effect on their serum calcium. Bomskov,⁵ Condorrelli,⁶ and Moritz,⁷ however, report that they were able to raise the serum calcium of rabbits by the injection of Parathormone. During the course of this work, the results of the latter authors could not be confirmed. Single or repeated injections of the active prin-

¹J. Biol. Chem. 88, 629 (1930).

²Medicine 5, 1 (1926).

³J. Biol. Chem. 68, 325 (1926).

⁴Parathormone is the name under which Eli Lilly Co. sells parathyroid extract.

⁵Klin. Wochenschr. 2, 2065 (1930).

⁶Bull. Soc. ital. di biol. sperm. 2, 250 (1927).

⁷J. Biol. Chem. 66, 343 (1925).

ciple of the parathyroid glands did not noticeably affect the serum calcium of rabbits. In Table VI are shown the results of repeated injections of Parathormone into normal adult rabbits. In no case was there any marked rise in the serum calcium concentration. As may be seen in the table, there was generally, after a period of several hours a drop in the serum calcium concentration. This drop was undoubtedly due to the repeated bleedings which the animals underwent. The data in Table VI show also that single doses of Parathormone were not effective in raising the serum calcium of rabbits.

In one of his papers, Collip¹ states that the oral administration of calcium to an animal, following the injection of parathyroid extract, was reflected in an increased response to the active principle. In following up this clue, it was found that if calcium chloride in large doses was given by mouth to a rabbit after an injection of Parathormone, high values for serum calcium concentration were obtained. However, it was also found that the serum calcium content of normal adult rabbits was temporarily elevated by merely giving a large dose of calcium chloride orally (Table VII).

This finding made it seem inadvisable to attempt to use simply a single injection combined with a single administration of calcium in order to show the presence of parathyroid hormone. From further work along this line, it developed that if the calcium, instead of being given in a single large dose, is given in several repeated small doses, the effect of each succeeding dose is minimized. Then if the serum calcium value obtained after each administration is plotted against the time of the bleeding,

¹Medicine 5, (1926).

it is seen that the peak occurs after the first administration of calcium, and regardless of further administrations, the calcium value tends to return to normal. However, if at the beginning of the series of calcium administrations, an injection of Parathormone above a certain minimal amount is given, the curve so obtained has its peak at three or five hours after the first administration of calcium, and the resulting calcium values are higher than in the case where no injection is given. By applying these findings, a method was developed which would show the presence in the material injected of 1.0 unit of Parathormone per kilogram of body weight of the test animal. The details of the method follow.

Adult rabbits, weighing about two kilograms, and kept on the Steenbock stock diet¹ for a period of four or five days preceding their use, are used for the test. The importance of keeping the animals on a standard diet such as the above cannot be overemphasized, for change of diet causes fluctuations in the serum calcium values of rabbits and makes the results of the test difficult of interpretation. It was found, however, that in fifty rabbits kept on the recommended diet the mean calcium value was 3.12 millimols per liter of serum, and that the standard deviation was 0.18 millimol.

When a test was to be performed, the animals were bled from the heart and immediately afterward the Parathormone was injected into the muscles of the rear legs. Immediately following the injection, ten cubic centimeters of a solution of calcium chloride containing 100 milligrams of calcium was given by stomach tube, and was washed down with an additional five cubic centimeters of water. One hour later another dose of calcium in the same

¹Steenbock, J. Biol. Chem. 80, 431 (1928).

amount and manner was given. Ten to fifteen minutes after this dose the animals were bled again. This procedure was repeated at three and five hours after the first bleeding. By the end of five hours each animal had received four doses of calcium chloride, had been bled four times, and had received one injection of Parathormone. The procedure for the controls was exactly the same with the exception that no injection was given.

The Parathormone, which comes in five cubic centimeter ampules and is assayed as 20 units per cubic centimeter, was measured as accurately as possible and injected with a tuberculin syringe. In those cases in which very small doses were given, the Parathormone was diluted with a measured amount of distilled water and an aliquot of the resulting solution taken, in order to increase the accuracy in measuring the amount injected.

The curves in Figure 3 are made from all of the cases observed. As can be seen, the peak of the curve for the controls appears at one hour, and the serum calcium values at three and five hours are either normal or on the way towards their initial value. In those cases in which 0.3 to 0.7 units of Parathormone per kilogram were injected, the results are, within the limits of error of the analysis, the same as those obtained with the control animals. The peak of the curve is at one hour, and at three and five hours the values are approaching normal. But when amounts of Parathormone in excess of 1.0 unit per kilogram of body weight are given, the shape of the curve is changed, the peak appearing at three or five hours instead of at one hour. Furthermore, the magnitude of the values for serum calcium concentration at the peak are much higher than the values at the peak of the curve summarizing those cases in which no Parathormone was given. From a further study of the figure, it appears that the higher the

dose of Parathormone the higher the values which are reached. This was true in general, but as can be seen from Table IX this did not always occur.

Table VIII shows the results of the repeated administration of 100 milligrams of calcium to normal adult rabbits without an accompanying injection of Parathormone. No value obtained at three or five hours exceeded 3.70 millimols of calcium per liter of serum, and only four of the forty-four were obtained in the range between 3.60 and 3.70 millimols per liter. The remaining forty values were below 3.60 millimols per liter at these times. In Table IX which gives the results obtained from the rabbits which had been injected with Parathormone, it is shown that all but three of the rabbits which received 1.0 to 4.0 units of Parathormone per kilogram of body weight, had serum calcium values which at three or five hours were higher than the highest value reached by any control at the same time. Seven of the fifteen cases had values of over 4.00 millimols per liter of serum, four were between 3.80 and 4.00, and one was just above those values which were reached by the controls. The effect of giving larger doses of Parathormone, although more marked in certain instances, was generally not much different than that of giving doses ranging from 1.0 to 4.0 units.

In analyzing these results, it is found that of the twenty-seven cases receiving more than 1.0 unit per kilogram of body weight, eighteen had values higher than 4.00 millimols per liter, four between 3.80 and 4.00 millimols per liter, and three between 3.50 and 3.70 millimols. Allowing an experimental error of 0.1 millimol of calcium per liter, which is much beyond the actual experimental error of the method, as will be shown later, and also for the possibility of finding a slightly higher value than the

ones actually found for the controls owing to individual peculiarities that might be encountered in a large number of animals, the conclusion may be drawn, that if in performing this test, values above 4.00 millimols per liter are obtained in a few cases, or values between 3.80 and 4.00 millimols are obtained in several cases, at the third or fourth bleeding, the amount of material injected has been equivalent in its effect to at least 1.0 unit of Parathormone per kilogram of weight of the test animal. Table X shows the distribution of the times at which the maximum for each experiment was reached, and Table XI summarizes the significant findings in the data presented in Tables VIII and IX.

This test was then applied to the solution of the problem which has previously been stated; namely, does the serum of rachitic rabbits, which has the faculty of taking up large amounts of calcium, contain an amount of parathyroid hormone greater than that found in normal rabbits, raised under the same conditions, except for the added cod liver oil in their diet?

A group of rabbits was made rachitic in the same manner as described previously. Another group on the same diet to which had been added cod liver oil in the amount stated above was used as a control. After a period of three to four weeks, when typical rickets had developed, the blood of the rachitic rabbits was tested for parathyroid hormone in the following manner.

An adult rabbit, having previously been kept on the Steenbock stock diet for four or five days, was bled from the heart, about seven to eight cubic centimeters of blood being drawn. The rachitic rabbit on whose blood the test was to be performed was then bled in a similar manner, and as much blood was taken out as possible. This was generally only about twenty cubic centimeters as the rabbits are quite small at this stage. This blood was

immediately injected intramuscularly in two places into the adult rabbit which was being used as the test animal. In all cases in which enough blood was obtained from the rachitic rabbit, two to three cubic centimeters were withheld in order to check the X-ray picture of the rickets by means of the determination of the inorganic phosphorus in the serum. Immediately after the blood from the rachitic rabbit had been injected into the test animal, 100 milligrams of calcium as calcium chloride in ten cubic centimeters of water was administered by stomach tube to the test animal and was washed down with an additional five cubic centimeters of water. At one, three, and five hours after the first treatment, another dose of calcium in the same amount and manner was given, and ten to fifteen minutes after each administration, seven to eight cubic centimeters of blood were drawn and the calcium concentration in the serum was determined.

The same procedure was followed in the case of the controls,--i.e. those rabbits which were placed on a rickets-producing diet to which cod liver oil had been added.

The results of this procedure are shown in Tables XII and XIII and in Figure 4. In Table XII, it is seen that of the twelve test rabbits into which rachitic blood was injected, in three cases the maximum serum calcium values at three or five hours were between 3.75 and 4.00 millimols per liter. In nine cases out of the twelve, the maximum values at three or five hours were above 4.00 millimols per liter. In all of the twelve cases the maximum values for the whole experiment were either at three or five hours. In other words, by the criteria previously established, out of twelve rachitic rabbits upon whose blood the test was performed, three values were obtained which are highly suggestive of the fact that parathyroid hormone was injected into the test

animals, and nine values of the twelve are higher than the extreme outside limits of error as determined previously for animals receiving no parathyroid hormone.

In the case of the normals (Table XIII), no value at three or five hours exceeds those obtained by giving to normal adult rabbits, without any injections, four doses of calcium at the time intervals employed in determining parathyroid hormone; and in all cases the maximum for the whole experiment was not at three or five hours but at one hour.

In Figure 4, the average values for the serum calcium content of the test animals at the times determined are shown together with the curves previously obtained with Parathormone injections accompanied by calcium administration, and with calcium administrations alone. It may be seen from this figure that the curve obtained when the test was performed on rachitic blood is very similar to the one previously obtained with injections of from 1.0 to 4.0 units of Parathormone. The curve obtained by the use of normal blood lies much below that obtained with rachitic blood, but slightly higher than the curve of the rabbits receiving no injections. This may mean that in normal blood there is a slight amount of parathyroid hormone, but the accuracy of the method described is not great enough to warrant such a conclusion. The fact that the deviation exists is interesting but not significant. At any rate, the data presented show rather conclusively that in the blood of rachitic rabbits there is a substance, which may or may not be parathyroid hormone, but which acts in a manner exactly similar to that in which parathyroid extract acts, in that it increases the amount of calcium which the blood of normal rabbits into which it has been injected can take up when calcium chloride is administered to these rabbits in the manner described.

DISCUSSION

It may be assumed rather safely from the results of this work and from the reports in the literature concerning the hypertrophy of the parathyroid glands in rickets, as determined histologically, that in rickets there is an actual hyperactivity of these glands. The significance of this hyperactivity cannot from the knowledge at hand be definitely determined, but on the basis of some rather conservative assumptions together with the data at hand, certain conclusions as to the significance of this hypertrophy may be drawn, which are at least in accordance with all of the facts known.

When Erdheim¹ published the first recorded work on hypertrophy of the parathyroid glands in bone diseases, he made the statement that this hypertrophy was compensatory and beneficial, and that it was found in rickets, osteomalacia, and osteitis fibrosa. Some years later it was definitely shown that in the case of osteitis fibrosa the hyperactivity of the parathyroid glands is not at all compensatory but is the primary cause of the condition.²

In view of the fact that Erdheim's contentions were shown to be wrong with reference to osteitis fibrosa, there was for a time the belief that his views regarding rickets and osteomalacia were also erroneous.³

¹Loc. cit.

²Mandl, Zentral. für Chir. 53, 260 (1926).

³Wilder, Endocrinology 13, 231 (1923).

In analyzing the situation in these conditions, it is found that in rickets and osteomalacia the calcium of the blood serum is quite normal or but slightly reduced. In the case of osteitis fibrosa the serum calcium is higher than normal; and since it has been definitely shown experimentally that injections of an excess of parathyroid hormone causes a condition similar to osteitis fibrosa,¹ accompanied by a high serum calcium, it may be assumed that when hypertrophy of the parathyroid glands is in part reflected by a high serum calcium, it is primary and causative.

But in rickets, there is not a high serum calcium, and yet there is a definite hypertrophy of the parathyroid glands. What then is the significance of this fact? It is known beyond reasonable doubt that for some reason, the animal organism during the development of rickets finds itself lacking utilizable calcium. Let it be assumed that this lack of utilizable calcium is reflected either by a tendency towards a lowering of the serum calcium concentration or even by an actual lowering. Let it further be assumed that this lowering of the serum calcium concentration might function as a stimulus to the parathyroid glands and activate them so that they maintain a normal or nearly normal blood calcium level by holding all of the available calcium in the blood, or even, if necessary, by dissolving it out of the bones. Thus it may be seen that the hypertrophy of the parathyroid glands in rickets seems on the basis of a few reasonable assumptions to be a compensatory hypertrophy in which the stimulus is a lack of utilizable calcium occasioning a tendency towards hypocalcemia. This state of affairs seems rather reasonable when one realizes how easily rachitic animals are thrown

¹Bodansky, Blair, and Jaffe, loc. cit.

into tetany. For obviously, if this hypertrophy is compensatory in nature and is dependent upon a tendency towards hypocalcemia, the balance between the calcium content of the organism and the secretion of the parathyroid glands, is very precarious indeed, and the slightest strain would tend to disturb this equilibrium and cause the animal to go into tetany.

From these considerations one would expect then, that rickets in animals would in a high degree be associated with latent tetany. That this is the case has been shown by Stoltenberg.¹ He found from the galvanic reactions of rabbits on a rickets producing diet, that during the first two months a large portion of the animals exhibited latent tetany. After a time, this number decreased. Hence, according to him, a rickets producing diet may produce pure rickets or rickets associated with active or latent tetany, depending upon the degree to which the parathyroid glands respond to the lack of calcium in the organism.

The fact that Wilder, who first inclined toward the belief that the hypertrophy of the parathyroid glands in rickets was a causative factor,² was able to prevent hypertrophy in rickets by administration of parathyroid extract to animals on a rickets producing diet, points very strongly to the compensatory nature of this hypertrophy.³

The assumption that a hypocalcemia in the organism is the stimulus which activates the parathyroid glands to hypertrophy, cannot of course be definitely shown, but the literature bears

¹Studier. over Eksperimental Raket, Monograph, J. W. Broktrypkeri, Bergen, 1928.

²Loc. cit.

³Higgins, Sheard, and Wilder, Proc. of the Staff Meetings of the Mayo Clinic 1, 311 (1932).

out the fact that this assumption is entirely reasonable. Thus it is well known that if the parathyroids of animals are extirpated, and the animals are kept alive by means of calcium medication, the organism gradually recovers from the resulting tetany and may live out its normal life span. This recovery is, on the basis of physiological grounds attributed to the hypertrophy of accessory parathyroid tissue.¹

Recapitulating briefly, it is seen, that on the basis of the facts that rachitic blood is capable of taking up more calcium than normal blood, that rachitic blood behaves in certain respects like parathyroid extract, that hypertrophy of the parathyroid glands has been demonstrated in rachitic animals, and that in rickets the animal organism lacks utilizable calcium; the single assumption that the parathyroid glands are stimulated to an increased activity by a tendency towards hypocalcemia, offers a logical and reasonable hypothesis, in accord with all of the known facts, for the role of the parathyroid glands in rickets.

¹Collip and Thompson, *Physiol. Rev.* 12, 309 (1932).

METHODS

The method used for the determination of phosphorus was that of Fiske and Subbarrow.¹ The pH of the serum was determined by the bicolorimetric method of Hastings and Sendroy.²

Calcium was determined by a modification of the method of Fiske and Logan.³ The details follow.

To two cubic centimeters of blood serum in a fifteen cubic centimeter centrifuge tube are added two cubic centimeters of a saturated solution of ammonium oxalate. The tube is stoppered up and placed in an ice box over night. On the following day, the precipitated calcium oxalate is separated by centrifugalization, and the supernatant liquid is poured off. The precipitate is washed twice with five cubic centimeters of 1% ammonium oxalate, the washing fluid being discarded and the precipitate being stirred up each time by twirling the centrifuge tube with the fingers. After the last washing the precipitate which forms a fine white pack on the bottom of the tube, is stirred up and dissolved in two or three drops of concentrated nitric acid, and washed into a platinum dish with about five two cubic centimeter portions of water delivered from a wash bottle. Two cubic centimeters of 2.5% oxalic acid are added and the whole evaporated to dryness on the steam bath. When the liquid has evaporated, the residue of calcium oxalate in the platinum dish is ignited at red heat over a Meker burner for about ten seconds, thereby con-

¹J. Biol. Chem. 66, 375 (1925).

²J. Biol. Chem. 61, 695 (1925).

³J. Biol. Chem. 93, 211 (1931).

verting the calcium oxalate into calcium oxide, which is titrated with 0.01 normal hydrochloric acid and methyl red as the indicator.

The accuracy of the method was determined by running forty-one parallel determinations on the blood from a single dog. One value obtained was 2.05 millimols per liter, which is an obvious error due perhaps to the difficulty of running so large a number of analyses in one day. The other values are shown in Table XIV. The mean is 2.34 millimols per liter, and the standard deviation is 0.029 millimol. Three times the standard deviation, or 0.09 millimol, was taken as the error of the method and used in the interpretation of the results.

SUMMARY

1. It has been shown that the blood of rachitic rabbits to which calcium is administered orally takes up this calcium to a far greater extent than does the blood of normal rabbits under the same conditions.

2. Rachitic rabbits administered calcium in sufficient doses generally die of hypercalcemia; the blood becomes viscous and clots very rapidly. Normal rabbits are not so affected.

3. A method has been presented for the determination of small amounts of parathyroid extract, normal adult rabbits being used as the test animals.

4. It has been shown that the blood of rachitic rabbits exhibits properties very similar to those of parathyroid extract, whereas the blood of normal rabbits does not.

5. A discussion of the role of the parathyroid glands in rickets has been presented.

TABLE I

Effect of Calcium Administration to Normal Rabbits

#	Mg. of Ca given	Salt	Serum Ca mM/liter		Serum inorganic P mM/liter		pH		Time sur- vived after admin- istra- tion
			Before	After	Before	After	Before	After	
147	50	chloride	2.95	3.20	1.88	1.90	7.38	7.30	>12 hrs*
141	50	"	2.90	3.60	1.72	1.84	7.40	7.20	>12 hrs.
15	100	gluconate	3.15	3.28	2.58	2.42	7.38	7.31	>12 hrs.
21	100	chloride	3.10	4.16	1.78	1.69	--	--	>12 hrs.
16	100	"	2.95	4.08	1.94	1.98	--	--	>12 hrs.
22	100	"	3.03	4.12	1.74	1.69	--	--	>12 hrs.
66	100	"	2.98	3.84	1.73	1.65	--	--	>12 hrs.
67	100	"	3.12	4.06	1.89	1.86	--	--	>12 hrs.
62	100	"	2.95	3.74	1.84	1.79	--	--	>12 hrs.
54	100	"	3.01	4.23	1.92	1.96	--	--	>12 hrs.
70	100	"	2.88	3.90	1.92	1.90	--	--	>12 hrs.
1	100	"	3.12	4.31	1.86	1.76	7.42	7.32	>12 hrs.
2	100	"	2.98	4.06	2.12	2.14	7.48	7.28	>12 hrs.
3	100	"	3.00	3.65	2.08	2.05	7.44	7.31	>12 hrs.
4	100	"	3.05	3.96	2.24	2.16	7.43	7.25	>12 hrs.
5	100	"	3.21	3.84	1.69	1.74	7.39	7.30	>12 hrs.
6	100	"	3.15	4.20	1.94	1.91	7.45	7.31	>12 hrs.
7	100	"	3.02	3.78	1.86	1.83	7.40	7.33	>12 hrs.
8	100	"	3.09	4.12	2.30	2.15	7.36	7.24	>12 hrs.
10	150	"	3.10	3.80	2.84	2.53	7.35	7.33	>12 hrs.
13	150	"	3.20	3.35	2.45	2.30	7.34	7.29	>12 hrs.
16'	200	gluconate	3.30	4.70	2.86	3.02	7.32	7.26	>12 hrs.
A3	200	chloride	2.95	3.72	2.46	2.49	7.36	7.32	>12 hrs.
Average			3.09	3.94	2.07	2.05	7.39	7.29	

*> = more than

TABLE II

Effect of Calcium Administration to Rachitic Rabbits

#	Mg. of Ca given	Salt	Serum Ca mM/liter		Serum inorganic P mM/liter		pH		Time sur- vived after admin- istra- tion
			Before	After	Before	After	Before	After	
49A	50	gluconate	2.95	4.40	0.94	0.92	7.35	7.30	>12 hrs.*
189	50	"	3.05	4.80	1.15	1.16	7.30	7.35	>12 hrs.
131	100	"	2.95	7.80	1.26	1.08	7.35	7.26	5 min.
99A	100	chloride	3.05	4.02	0.97	0.92	7.32	7.28	>12 hrs.
49B	100	"	2.95	4.80	0.74	0.73	7.33	7.33	>12 hrs.
75	100	"	3.10	3.55	0.78	--	--	--	>12 hrs.
69	100	"	3.06	3.98	0.73	--	--	--	>12 hrs.
93A	100	"	3.02	7.21	0.64	0.68	--	--	5 min.
78	100	"	2.92	3.15	0.22	--	--	--	>12 hrs.
80	100	"	2.98	3.35	0.26	--	--	--	>12 hrs.
348	100	"	3.00	7.40	0.72	0.69	--	--	15 min.
388	100	"	2.98	4.90	0.76	0.79	--	--	1 hr.
392	100	"	3.06	15.30	0.58	0.54	--	--	5 min.
393	100	"	3.00	5.20	0.61	0.65	--	--	2 hrs.
898	100	"	3.03	5.16	1.02	1.06	--	--	10 min.
899	100	"	2.96	5.72	0.73	0.68	--	--	10 min.
901	100	"	3.00	6.01	0.84	0.80	--	--	1 hr.
904	100	"	3.01	5.50	0.92	0.94	--	--	1 hr.
907	100	"	2.94	7.32	0.73	0.68	--	--	5 min.
911	100	"	2.97	5.68	0.90	0.84	--	--	>12 hrs.
61	100	"	2.95	5.31	1.03	0.98	--	--	1 hr.
49	100	"	2.95	8.31	0.98	1.01	--	--	1 hr.
53	100	"	3.01	5.82	1.02	0.94	--	--	1 hr.
69A	100	"	2.98	5.31	0.69	0.74	--	--	2 hrs.
62	100	"	3.01	6.42	0.98	0.84	--	--	2 hrs.
93B	100	gluconate	2.60	11.50	1.06	2.15	7.26	7.00-	5 min.
76	100	chloride	2.75	8.05	0.83	1.57	7.39	7.00	5 min.
2	150	"	2.70	9.05	1.27	1.29	7.36	7.30	15 min.
6	150	"	2.85	6.10	0.78	1.62	7.38	7.12	1 min.
99B	150	"	3.02	3.60	0.98	1.01	7.33	7.28	>12 hrs.
92	150	gluconate	3.09	7.85	0.58	0.66	--	--	5 min.
1	200	"	3.15	12.00	1.24	1.22	--	--	5 min.
69	200	"	2.96	9.03	0.54	--	--	--	5 min.
Average			2.97	6.47	0.86	0.88	7.34	7.30	

*> = more than

TABLE III

Summary of Effect of Calcium Administration on Serum Calcium of
Normal and Rachitic Rabbits

Serum Ca mM/liter	Number of rabbits having values in first column			
	Normal		Rachitic	
	Before	After	Before	After
2.69 to 3.39	23	3	33	2
3.40 to 3.99		10		3
4.00 to 4.49		9		2
4.50 to 4.70		1		
4.71 to 4.99				3
5.00 to 5.99				8
6.00 to 6.99				3
7.00 to 7.99				5
8.00 to 8.99				2
9.00 to 9.99				2
10.00 to 16.00				3

TABLE IV

Effect of Injection of Rachitic Rabbit Blood into Dogs

#	Substance injected	Serum Ca before injection	Serum Ca at various hours after injection							Maximum rise
			10	12	15	18	19	23	31	
1	Trichloroacetic acid extract of 40 cc. of rachitic rabbit blood	2.65			3.30	2.60				0.75
1	50 cc. of rachitic rabbit blood	2.35	2.75	3.38	2.98					0.63
2	50 cc. of rachitic rabbit blood at 0, 11, 15, and 19 hours	2.48	2.80		2.25		2.75	2.55	2.45	0.32
1	25 cc. of rachitic rabbit blood every hour for 6 hours	2.75	3.02		3.15					0.40
1	Trichloroacetic acid extract of 100 cc. of rachitic rabbit blood	2.70	2.75	2.70						0.05

TABLE V

Effect of Injection of Normal Rabbit Blood and of Parathormone
into Dogs

#	Substance injected	Serum Ca before injec- tion	Serum Ca at various hours after injection							Maxi- mum rise
			10	12	15	18	19	23	31	
1	50 cc. of normal blood at 0, 11, 15, and 19 hours	2.60	2.65		2.35		2.25	2.40	2.50	0.05
2	40 cc. of normal blood	2.40	2.38		2.50	2.40				0.10
1	Trichloracetic acid extract of 50 units of Parathormone	2.60	3.50		3.90	3.45				1.30
2	50 units of Parathormone	2.25	3.00	3.10	3.55					1.30

TABLE VI

Effect of Single and Repeated Injections of Parathormone
on Normal Adult Rabbits

(The figures are millimols of Calcium per liter of serum.)

Experiment	1	2	3	4	5	6	7	Controls	
Hour 0	3.05 50 u*	20 u	3.10 30 u	3.20 20 u	3.55 5 u	3.45 10 u	3.25 10 u	3.40	3.08
1	3.15	3.13	3.20						3.12
2	3.05	3.15	3.20		5 u	10 u	10 u		3.40
3	4.20			3.25					3.56
4	3.53	3.10	3.15		5 u	10 u	10 u		3.05
5	2.95	3.20	2.85						3.08
6		3.20	2.95		5 u	10 u	10 u		
8	3.00	3.15	2.40		5 u	10 u	10 u		2.96
10					3.65	3.90	3.20	3.20	
12					3.45	3.45	3.25	2.98	
14					2.98	3.18	2.75	2.83	
16					2.70	2.90	2.45	2.83	
18					2.78	2.96	2.45	2.75	
20					Died	2.90	2.38	Died	
22						Died	2.45		
24							Died		

*u means units of Parathormone

TABLE VII

Effect of a Single Injection of Parathormone Combined with
a Single Large Dose of Calcium Chloride, and of a Single
Large Dose of Calcium Chloride on Normal Adult Rabbits

(The figures are millimols of Calcium per liter of serum.)

#	Hour: 0	1	2	3	4	
16	3.30 50 u*	---	---	---	1500 mg. CaCl_2 7.90	
100	2.85 75 u	---	---	---	2000 mg. CaCl_2 8.00	
91	3.30 3 u	---	---	---	500 mg. CaCl_2 5.06	
412	3.09 5 u	---	---	---	500 mg. CaCl_2 3.75	
461	3.25 4 u	---	---	---	500 mg. CaCl_2 4.43	
412	3.36	---	---	---	500 mg. CaCl_2 4.87	
461	3.30	---	---	---	500 mg. CaCl_2 5.83	
99	3.25	---	---	---	1500 mg. CaCl_2 5.12	

*u means units of Parathormone

TABLE VIII

Effect of Four Doses of 100 Milligrams of Calcium
to Normal Rabbits at 0*, 1, 3, and 5 Hours

#	Wt.(g.)	0 hr.	1 hr.	3 hrs.	5 hrs.
A305	1810	2.95	3.35	3.20	3.05
A171	1466	2.75	3.50	3.25	3.00
A284	1511	3.00	3.40	2.80	2.82
A133	1706	2.80	3.02	2.95	2.97
3	1579	3.32	4.02	3.58	3.25
1	1551	2.85	3.10	3.25	3.12
2	1427	2.75	3.10	3.50	2.85
26	2925	3.30	4.08	3.22	3.28
35	2611	2.75	3.67	3.39	3.44
37	2151	3.24	3.86	3.38	3.32
36	2371	3.20	3.68	3.59	3.51
55	2506	3.18	3.40	3.15	3.10
34	2794	3.15	3.25	3.00	2.85
26	2922	3.00	3.62	3.05	3.05
61	2246	3.30	3.25	3.35	3.03
M10	2629	3.20	3.55	3.55	3.00
63	1981	3.08	3.25	3.55	3.45
M10	2640	2.88	3.55	3.30	3.15
938	1641	3.10	3.45	3.48	3.13
61	2263	2.90	3.57	3.00	3.20
M10	2635	2.95	3.35	3.25	3.10
A530	1851	3.15	4.04	3.50	3.28
A569	2240	3.26	3.75	3.43	3.08
A673	2108	3.30	3.75	3.61	3.13
A700	1762	3.15	3.50	3.12	3.20
A704	1926	3.38	4.40	3.51	3.54
54	2376	3.25	4.00	3.48	3.31
60	2308	3.31	4.12	3.56	3.48
A700	1762	3.10	3.98	3.40	3.30
A483	----	3.31	3.75	3.49	3.26
A673	2108	3.25	4.18	3.64	3.48
A704	1903	3.25	3.80	3.00	3.31
A552	1632	3.15	3.87	3.60	3.40
A412	1861	3.26	4.08	3.39	3.25
A673	2108	3.00	3.76	3.42	3.36
A575	1874	3.40	4.10	3.52	3.50
A100	1651	3.01	3.79	3.51	3.38
A521	2110	3.32	3.85	3.40	3.24
94	1950	3.25	4.25	3.62	3.48
81	2495	3.07	3.08	2.98	3.25

*0 hr. in all cases means the beginning of the experiment. The following figures mean the time in hours after the 0 hour or beginning.

TABLE IX

Effect of One Injection of Parathormone at the 0 Hour,
and Four Doses of 100 Milligrams of Calcium at 0, 1,
3, and 5 Hours upon Normal Rabbits

#	Wt.(g.)	Units	0 hr.	1 hr.	3 hrs.	5 hrs.
304	1828	1	3.20	3.55	3.25	3.40
339	1803	1	2.90	3.23	3.15	3.20
245	----	1	3.25	3.55	3.28	3.33
A305	1810	1	3.05	3.65	3.45	3.00
A269	----	1	2.80	3.77	3.30	2.95
A339	----	1	3.05	3.70	3.35	3.25
84	1457	0.6	3.18	3.40	3.35	3.48
76	1279	0.8	3.13	3.82	3.45	3.05
311	1620	1	2.94	3.76	3.22	3.01
790	2114	1.3	3.10	3.73	3.60	3.00
91	1120	0.5	2.98	3.54	3.49	3.10
98	1896	1.3	3.00	3.44	3.40	3.15
78	1485	0.8	3.20	3.69	3.31	3.08
1	1548	2	3.00	3.25	3.23	3.90
A151	1624	2	3.20	3.30	3.58	3.45
A171	1481	2	2.75	3.45	3.55	3.35
2	1428	2	2.95	3.05	4.57	4.70
A133	1720	2	2.90	3.75	3.90	3.75
946	1823	1.8	3.25	3.85	4.12	3.94
A284	1514	5	3.25	3.35	3.73	4.10
A133	1731	5	3.00	3.05	4.05	3.60
A171	1470	5	3.15	3.32	3.70	3.35
1	1560	5	3.05	3.10	4.05	4.00
2	1425	5	2.70	3.40	3.75	4.12
50	2590	10	3.18	3.65	3.70	4.03
35	2672	10	3.12	3.30	3.60	2.50
54	2876	10	3.35	3.80	3.85	3.43
M10	2635	10	3.10	3.70	3.95	3.35
3	1565	10	3.12	4.25	4.45	5.20
50	2585	25	3.40	3.75	4.12	6.37
49	2416	20	3.10	6.90	4.20	Died
34	2793	20	3.30	4.01	4.28	3.84
38	2712	20	3.28	3.98	4.08	3.59
35	2685	20	3.30	3.50	4.30	4.25
53	2861	20	3.35	3.70	3.70	3.30
26	2930	20	3.30	3.55	3.70	4.70
938	1622	15	3.03	3.30	4.10	4.15
60	2308	50	3.30	3.45	4.05	3.95
A100	1651	50	2.75	4.10	4.20	4.75
129	2560	25	3.25	3.53	3.90	4.20
88	2310	50	3.15	3.75	4.50	4.35

TABLE X

Each figure shows the number of rabbits having their maximum value for serum calcium concentration at the time indicated at the left:

Time	Controls	Parathormone per kilogram		
		0.3-0.7 units	1.0-4.0 units	6.0-32 units
1 hour	34	12	0	1
3 hours	5	0	10	6
5 hours	1	1	5	6

TABLE XI

Summary of Tables VIII and IX

Highest serum Ca at 3 or 5 hrs. (in mM/liter)	Controls	Parathormone per kilogram		
		0.3-0.7 unit	1.0-4.0 units	6.0-32 units
less than 3.00	2			
3.00-3.09	2			
3.10-3.19	1			
3.20-3.29	8	2		
3.30-3.39	5	5		
3.40-3.49	8	5		
3.50-3.59	10		2	
3.60-3.69	4	1	1	
3.70-3.79			1	1
3.80-3.89			1	
3.90-3.99			3	
4.00-4.09			3	2
4.10-4.19			3	1
4.20-4.29				3
4.30-4.39				1
4.40-4.49				
4.50-4.59				1
4.60-4.69				
4.70-4.79			1	2
4.80-4.89				
4.90-4.99				
5.00-5.09				
5.10-5.19				1
6.37				1

TABLE XII

Test for Parathyroid Hormone in the Blood of Rachitic Rabbits
(Values in millimols per liter of serum.)

#	Wt. (g.)	Rachitic whole blood injected (cc.)	P con- tent	S e r u m C a l c i u m				Max. at 3-5 hrs.	Time of max. for whole exper- iment
				0 hr.	1 hr.	3 hrs.	5 hrs.		
B144	2013	8	--	3.31	3.50	3.75	3.61	3.75	3 hrs.
B151	1980	12	--	3.25	3.53	3.58	3.81	3.81	5 hrs.
B153	1710	13	--	3.26	3.98	4.06	3.94	4.06	3 hrs.
576A	1943	13	1.03	3.35	4.03	4.10	4.25	4.25	5 hrs.
B154	1850	14	0.78	3.38	3.95	4.03	3.64	4.03	3 hrs.
W1	1925	15	1.24	3.24	3.75	4.10	3.57	4.10	3 hrs.
A483	1741	15	0.69	3.10	3.62	3.88	3.41	3.88	3 hrs.
B50	1920	16	0.98	3.18	3.54	4.02	3.52	4.02	3 hrs.
154A	1850	18	--	3.30	3.58	3.94	4.01	4.01	5 hrs.
622	1635	18	1.02	3.23	3.61	4.01	3.95	4.01	3 hrs.
144A	2030	19.5	--	3.25	3.55	4.13	3.68	4.13	3 hrs.
576	1940	22	--	3.10	3.50	4.40	3.80	4.40	3 hrs.

TABLE XIII

Test for Parathyroid Hormone in the Blood of Normal Rabbits

(Values in millimols per liter of serum.)

#	Wt. (g.)	Normal whole blood injec- ted (cc.)	P con- tent	S e r u m C a l c i u m				Max. at 3-5 hrs.	Time of max. for whole exper- iment
				0 hr.	1 hr.	3 hrs.	5 hrs.		
A412	1730	12	1.94	3.24	3.85	3.43	3.31	3.43	1 hr.
153A	1710	12	1.86	3.23	3.48	3.30	3.32	3.32	1 hr.
B166	1903	13	1.92	3.25	3.86	3.54	3.32	3.54	1 hr.
151A	1846	14	1.92	3.25	3.71	3.58	3.43	3.58	1 hr.
B4	1719	15	--	3.10	4.01	3.62	3.38	3.62	1 hr.
A571	1886	15	1.74	3.20	3.85	3.55	3.38	3.55	1 hr.
A607	1841	15	1.78	3.06	3.97	3.61	3.40	3.61	1 hr.
144B	2013	15	2.05	3.30	3.61	3.38	3.30	3.38	1 hr.
M6	2640	15	1.74	3.18	3.74	3.49	3.35	3.49	1 hr.
151A	1980	16	1.92	3.25	3.52	3.46	3.35	3.46	1 hr.

TABLE XIV

Distribution of Results of Forty Parallel Analyses for Calcium in
Dog Serum

Calcium in mM/liter:	2.25-2.28	2.29-2.32	2.33-2.36	2.37-2.40
Determinations:	3	4	26	7
Mean: 2.34 mM/liter				
Standard deviation: 0.029 mM/liter				

Figure 1. Effect of Administration of Soluble Calcium Salts
to Normal and Rachitic Rabbits

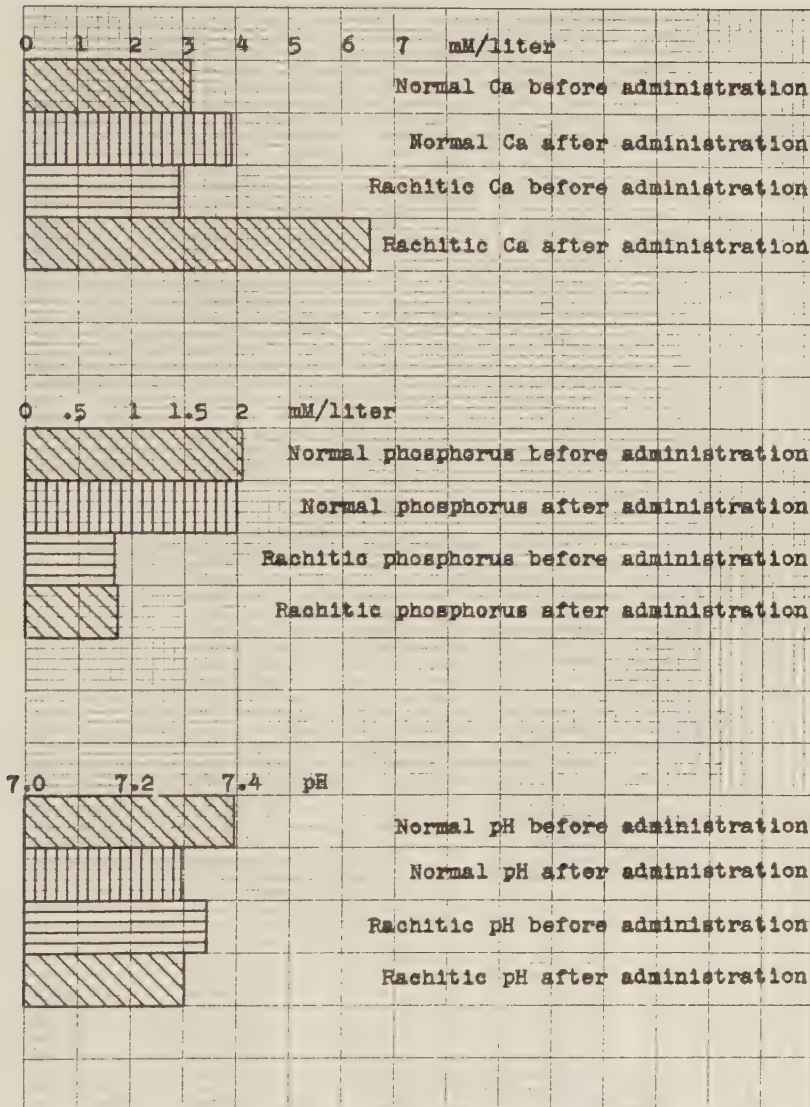


Figure 2. Average Maximum Rise in Serum Calcium of Dogs with Injections of Normal Blood, Rachitic Blood, and Parathormone

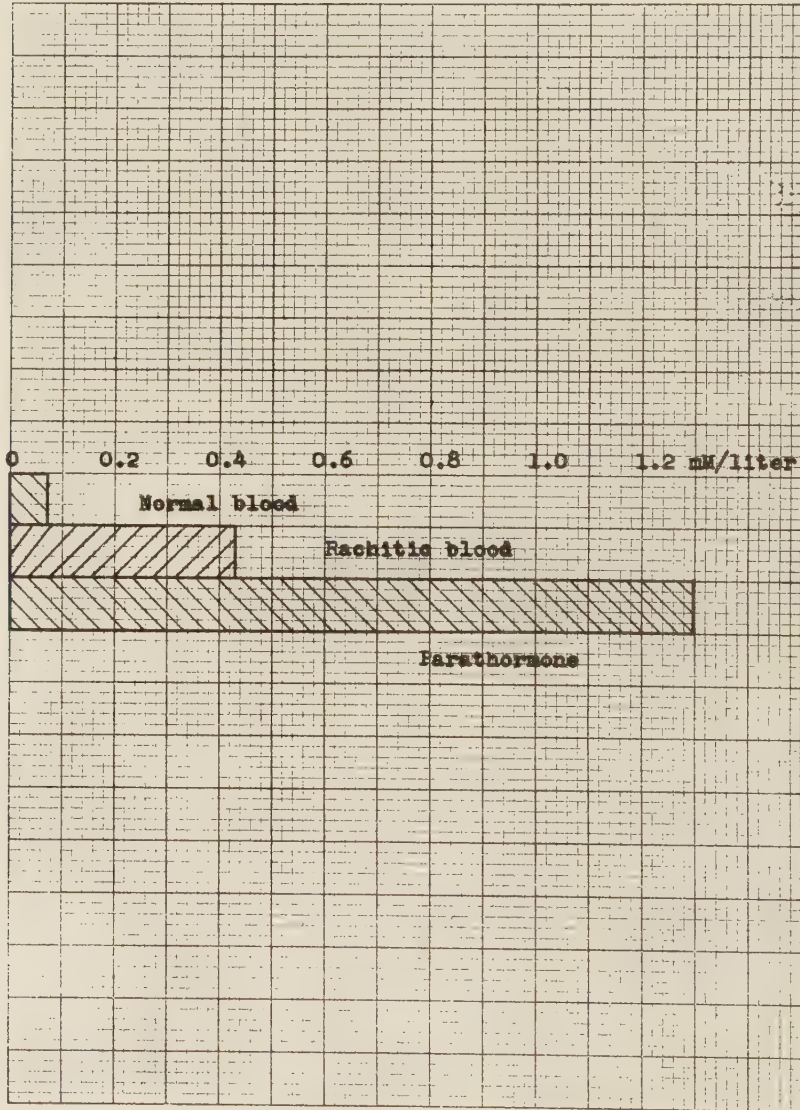


Figure 3. Summary of Tables VIII and IX

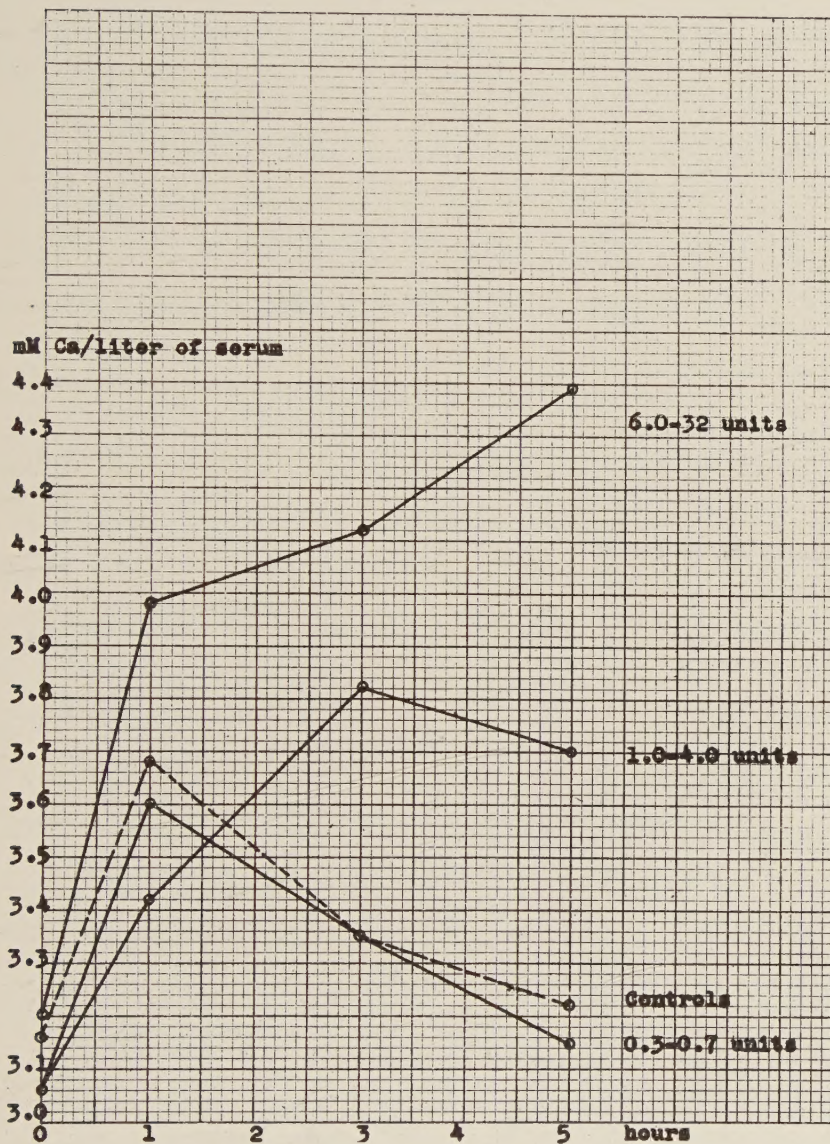


Figure 4. Summary of Tables XII and XIII

